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Can activation of NRF2 be a strategy against COVID-19?

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Keywords

anti-inflammatory, ARDS, bardoxolone methyl, KEAP1, SARS-CoV-2, sulforaphane

Abstract

Acute respiratory distress syndrome (ARDS) caused by SARS-CoV-2 is largely the result of a dysregulated host response, followed by damage to alveolar cells and lung fibrosis. Exacerbated pro-inflammatory cytokines release (cytokine storm) and loss of T-lymphocytes (leucopenia) characterize the most aggressive presentation. Here we propose that a multi-faceted anti-inflammatory strategy based on pharmacological activation of nuclear factor erythroid 2 p45-related factor 2 (NRF2), can be deployed against the virus. The strategy provides robust cytoprotection by restoring the redox and protein homeostasis, promoting resolution of inflammation, and facilitating repair. NRF2 activators such as sulforaphane and bardoxolone methyl are already in clinical trials. The safety and efficacy information of these modulators in humans, together with their well-documented cytoprotective and anti-inflammatory effects in preclinical models, highlight the potential of this armamentarium for deployment to the battlefield against COVID-19.

Exacerbated inflammation in severe COVID-19 pathology

Numerous clinical observations during the outbreaks of coronaviruses severe acute respiratory syndrome coronavirus (SARS-CoV), Middle East respiratory syndrome-related coronavirus (MERS-CoV), and most recently, SARS-CoV-2, convincingly show that, in addition to virus propagation, the host inflammatory response is a crucial determinant of disease outcome [1]. A parallel can be drawn with influenza, for which lethality is not associated with the cytolytic action of the pathogen, but rather with the inflammatory response orchestrated by the host immune system [2]. In the most severe cases of the disease, a cytokine storm (excess production of cytokines) [3] is associated with T cell depletion, pulmonary inflammation, and lung damage. Patients showing **acute respiratory distress syndrome** (**ARDS**; see Glossary) and other types of virally-induced pneumonia also present features of **macrophage activation syndrome** (**MAS**) [3]. There is also evidence of leukopenia, with a ~2-fold decrease in T cell number [4], which may be the result of pyroptosis, a form of cell death affecting mainly cells of the immune system [5]. On the other hand, **granulocytosis** might be partly responsible for the strong burst of superoxide [2], a type of **reactive oxygen species** (**ROS**) [6], and the additional production of pro-inflammatory cytokines [7].

In order to comprehensively manage the symptoms of COVID-19 (the disease caused by SARS-CoV-2), it is critical to understand the most appropriate context for introducing an anti-inflammatory therapy to complement an anti-viral therapy. Such therapy must control inflammation without altering the ability of the host to mount an efficient adaptive immune response against the virus. Here we propose that boosting the endogenous cellular defenses by targeting the cytoprotective transcription factor Nuclear factor erythroid 2 p45-Related Factor 2 (NRF2, gene name *NFE2L2*) will

promote the resolution of COVID-19 associated inflammation whilst in parallel, restore redox homeostasis and facilitate tissue repair. It should be noted that the protein under discussion is distinctly separate from the identically abbreviated Nuclear Respiratory Factor 2 (also known as GA Binding Protein Transcription Factor Subunit Beta, gene name *GABPB1*), which is a completely different transcription factor involved in mitochondrial biogenesis [8].

Overview of NRF2 and its anti-inflammatory roles

NRF2 is a cap´n´collar (CNC) transcription factor that heterodimerizes with small musculoaponeurotic fibrosarcoma (sMAF) proteins, K, G or F [9], or with transcription factors C-JUN and JUND [10], to bind to **Antioxidant Response Elements (AREs)** and regulates transcription of target genes, including those encoding proteins involved in cellular redox homeostasis, detoxification, macromolecular damage repair, and metabolic balance [11]. Under basal conditions, NRF2 interacts with the E3 ligase substrate adapter Kelch-like ECH-associated protein 1 (KEAP1) that targets the transcription factor for ubiquitination and proteasomal degradation [11-13] (Figure 1). **Electrophiles** and ROS (collectively termed inducers) inactivate KEAP1 by modifying specific sensor cysteine residues [14], resulting in NRF2 accumulation and enhanced target gene transcription.

NRF2 activity is frequently dysregulated in disease states, including diabetes, liver disease, and inflammatory bowel disease [15], and declines with aging [16]. Presence of some of these disease states (such as diabetes) and older age are some of the risk factors also associated with SARS-CoV-2-induced ARDS [17]. Importantly, activation of NRF2 has been shown to be involved in preserving lung architecture in response to inflammatory cues and therapeutic effects of NRF2 activation have been

reported in animal models of a number of lung disorders, including respiratory infections and ARDS [18]. Moreover, single nucleotide polymorphisms (SNPs) located in the promoter region of *NFE2L2* (encoding NRF2) have been implicated in lung disease susceptibility in humans, hence reinforcing NRF2 as therapeutic target for pulmonary diseases [19, 20].

NRF2 also plays a role in both the execution and the resolution of inflammation [12] by repressing proinflammatory genes, such as *IL-6* and *IL-1β* [21]. This is particularly prominent in lipopolysaccharide (LPS)-stimulated macrophage cells, where the anti-inflammatory immunometabolite itaconate that accumulates during the metabolic reprogramming of these cells, activates NRF2 [22]. Moreover, NRF2 also induces the transcription of several macrophage-specific genes that participate in tissue repair. These include macrophage receptor with collagenous structure (MARCO), a receptor required for bacterial phagocytosis, cluster of differentiation 36 (CD36), a scavenger receptor for oxidized low-density lipoproteins (LDL) [24], and IL-17D [25], which confer protection against viral infections [26]. Similarly, NRF2 activation restores redox homeostasis by upregulating **glutathione** (**GSH**), NADPH, thioredoxin, thioredoxin reductase, and peroxiredoxin that protect against **oxidative stress** and favor alternative wound healing *vs.* classical proinflammatory activation of macrophages and other immune cells [27].

NRF2 in viral infections

The role of NRF2 in viral infections has been investigated in the context of both DNA and RNA viruses. In general, viruses can benefit from either activating or inhibiting NRF2 in the host cells [28]. This might be dependent on factors such as the stage of infection [29], or to the specific mechanisms of viral propagation that favor

either death of the infected cells and lytic release of virions, or survival of the infected cells with reduction of the inflammatory response to help viral propagation [30]. In human coronavirus HCoV-229E, which is associated with common cold involved in pulmonary disease [31], deficiency in expression of the NRF2-target glucose-6-phosphate dehydrogenase (*G6PDH*) increases ROS production and enhances viral gene expression and particle production [32]. Crucially, the NRF2 pathway has been found to be suppressed in lung biopsies from COVID-19 patients, conversely pharmacological inducers of NRF2 inhibit the replication of SARS-CoV2 and the inflammatory response [33].

Interestingly, it is known that there is also a reciprocal crosstalk between NRF2 and NF-κB in inflamed tissues, where innate immune cells are recruited [34-36]. It has been shown that following infection with SARS-CoV, NF-κB is activated in lungs of mice and in human monocyte macrophages *in vitro*; conversely, inhibition of NF-κB decreases inflammation and improves survival after SARS-CoV infection in mice [7, 37]. Thus, pharmacological activation of NRF2 might also limit the NF-κB-mediated inflammatory processes inflicted in the lung by SARS-CoV-2 infection.

SARS-CoV-2 biology and potential crosstalk with NRF2

The SARS-CoV-2 genome encodes non-structural proteins (nsp) required for replication, structural proteins including spike (S), envelope (E), membrane (M), and nucleocapsid (N), and accessory proteins orf 3, 6, 7a, 7b, 8 and 9b that interact with the host cells [38]. The receptor binding domain (RBD) located in the S protein of SARS-CoV-2 interacts with the **angiotensin-converting enzyme 2** (**ACE2**) of host cells to allow viral entry (**Figure 1, Key Figure**) [39]. The use of ACE inhibitors/angiotensin-receptor blockers, which are widely prescribed to patients with cardiovascular

pathologies [40], is currently being considered for COVID-19 (Clinical Trial Number[i]: NCT04311177 and NCT04312009 for the use of Losartan) as angiotensin II, the target of ACE inhibitors, has vasoconstrictive, proinflammatory, pro-oxidative and pro-thrombotic effects [41]. However, these inhibitors alter the balance ACE/ACE2 and increase ACE2 levels, thus potentially increasing the number of docking sites for viral entry [42]. NRF2 deficiency is known to upregulate ACE2 and its activator oltipraz reduces ACE2 levels [43], suggesting that NRF2 activation might reduce the availability of ACE2 for SARS-CoV-2 entry into the cell (**Figure 1**).

By analogy with other coronaviruses, SARS-CoV-2 is expected to modulate the host translational machinery in order to favor generation of its own proteins (Figure 1) [44]. Host countermeasures to this step include the inactivation of eukaryotic Initiation Factor 2 (eIF2) by two of the three cellular eIF2α kinases, protein kinase R (PKR) and PKR-like endoplasmic reticulum kinase (PERK), which are known to be activated in response to SARS-CoV infection [45]. Interestingly, PKR also has the potential to upregulate the autophagy cargo protein p62, which competes with NRF2 for binding to KEAP1 [46] and further promotes the autophagic degradation of KEAP1 [47], thus activating NRF2 transcriptional activity (**Figure 1**). Moreover, it has been observed in SARS-CoV that host-induced blockade of translation of coronavirus proteins, including the S protein, triggers the **unfolded protein response (UPR)**, activating PERK [48] that phosphorylates and activates NRF2 [49]. This can then be one step when NRF2 can be modulated to reduce the potential of SARS-CoV-2 infections in host cells.

Cells infected with RNA viruses recognize viral molecular patterns, especially nucleic acids, by cytoplasmic and endosomal receptors, such as the RNA sensors retinoic acid-inducible gene I (RIG-I) and melanoma differentiation-associated protein 5 (MDA-5) [50] and the DNA sensor cyclic GMP-AMP Synthase (cGAS), which signals

through the adaptor protein **STimulator of INterferon Genes** (**STING**), to mediate appropriate immune response [51]. These innate detection systems turn on Interferon Regulatory Factor 3 (IRF3)-mediated transcription of type I and III interferons (IFNs) [51]. Coronavirus infections (including SARS-CoV-1) have been shown to antagonize STING mediated host immune systems [52, 53]. While Type I IFNs are crucial for restricting viral replication and spread through autocrine and paracrine type I IFN receptor signaling, excessive release of IFNs by infected pulmonary alveolar cells or resident macrophages may exacerbate the pulmonary infiltration of additional monocyte-derived macrophages, further potentiating inflammatory damage [54]. NRF2 downregulates IFN production, in part by downregulating STING expression [55, 56] (**Figure 1**). Therefore, NRF2 may attenuate the inflammatory response to viral infection by preventing excessive production of IFNs.

Additionally, upregulation of the NRF2-transcriptional target heme oxygenase 1 (HO-1, gene name *HMOX1*) has been linked to an antiviral response against many viruses including HIV, hepatitis C virus (HCV), hepatitis B virus (HBV), enterovirus 71 (E71), influenza virus, respiratory syncytial virus (RSV), Dengue virus (DENV) and ebola virus (EBOV) [57]. HO-1 can mediate antiviral responses by forming a heterodimeric complex with IRF3 [58]. With this interaction, IRF3 is phosphorylated and translocated into the nucleus where it induces the expression of type I IFNs (**Figure 2**).

Several other mechanisms have been described for the control of viral infection by HO-1, which can be extrapolated to some extent to SARS-CoV-2. HO-1 catalyzes the degradation of heme into three products, biliverdin, Fe^{2+} and CO, each one having putative anti-SARS-CoV-2 activity. Coronaviruses produce viral proteases, 3C-like proteinase (3CL-pro) and papain-like protease (PLpro) that process the viral

polyproteins and are essential for viral replication [59]. Both 3CL-Pro and PLpro share high homology with other viral proteases [60], which are known to be inhibited by biliverdin [61] (**Figure 2**). Biliverdin is then expected to inhibit SARS-CoV-2 3CLpro and PLpro too. Free Fe^{2+} binds to the highly conserved divalent metal-binding pocket of RdRp of HCV, inhibiting its enzymatic activity (**Figure 2**) [62, 63]. Since this binding pocket is highly conserved in SARS-CoV-2 [64] a similar mechanism may confer a NRF2/HO-1 mediated antiviral activity in COVID-19. Furthermore, CO elicits antiviral responses against +ssRNA viruses such as E71 [65] and Bovine Viral Diarrhea Virus (BVDV9) [66] and this effect is phenocopied by the CO donor, CO releasing molecule-2 (CORM-2) through a mechanism dependent on the activation of soluble guanylyl cyclase (sGC), which increases the local levels of cyclic guanosine monophosphate (cGMP) and activates protein kinase G (PKG) (**Figure 2**). In turn, PKG inhibits NADPH oxidases (NOX) [67], preventing an increase in ROS levels (**Figure 2**) that otherwise would contribute to inflammation. If these mechanisms are also mirrored in the context of COVID-19, activation of the NRF2/HO-1 pathway holds promise for mitigating SARS-CoV-2 infection.

Armamentarium of available NRF2 activators for potential anti-inflammatory therapy of COVID-19

An important limitation for development of effective therapies against SARS-Cov-2 is the poor reproducibility of COVID-19 in animal models, most of which either do not share relevant physiology, do not mount an appropriate immune response, or do not present relevant clinical symptoms [68]. Nevertheless, genetic or pharmacological NRF2 activation has consistently demonstrated anti-inflammatory and anti-viral effects in other pathologies in animals and in humans. The most physiologically and

pharmacologically relevant mechanism of NRF2 regulation is by targeting specific cysteine sensors within KEAP1 [11]. A comprehensive review on the use of NRF2 activators against viral infections has been published recently [69]. Considering that changes in redox homeostasis in infected cells and lung inflammation are hallmarks of infections caused by respiratory viruses [70], the information obtained from viruses that affect the airways may be relevant for extrapolation to COVID-19. Indeed, experimental evidence is beginning to emerge, and it was recently demonstrated that the NRF2 activators dimethyl fumarate (DMF) and 4-octyl itaconate (4-OI), a cell permeable analog of the endogenous anti-inflammatory metabolite itaconate [22], suppress the inflammatory response to SARS-CoV2 in human cells, including peripheral blood mononuclear cells (PBMCs) from COVID-19 patients [33].

The NRF2 activators targeting KEAP1 that are under clinical development have been recently described [11, 71]. Here, we discuss DMF, the only drug approved by Food and Drug Administration (FDA) and European Medicines Agency (EMA) that targets the NRF2/KEAP1 axis [72], along with two types of NRF2 activators which have been tested in advanced clinical trials (Table 1), and thus can be immediately expedited to clinical trials to examine their therapeutic efficacy in patients with COVID-19.

Dimethyl fumarate (DMF)

DMF is used in the treatment of multiple sclerosis (MS) and psoriasis and may have beneficial effect in lung diseases [73]. Consistent with its role in prevention of de-myelination, DMF protects against Theiler's Murine Encephalomyelitis Virus (TMEV), a +ssRNA that causes a MS-like disease, via enhancing the NRF2 antioxidant and anti-inflammatory responses as well as suppressing IL-17A [74]. However, a well-characterized off-target effect, not related to the NRF2/KEAP1 axis, is leukopenia that

occurs in a subset of MS patients [75]. Considering that leukopenia is a hallmark of severe cases of COVID-19, the potential use of DMF in this setting should be considered with caution.

Sulforaphane

The isothiocyanate sulforaphane (SFN), originally isolated from broccoli, a cruciferous vegetable, as an inducer of the classical NRF2 target, **NAD(P)H:quinone oxidoreductase 1** (**NQO1**) [76], is the most potent naturally occurring NRF2 activator, with well-documented antioxidant and anti-inflammatory effects [77]. The high **bioavailability** of SFN and its stabilised α-cyclodextrin encapsulated version sulforadex (SFX-01), makes it an excellent candidate for alleviating excessive anti-inflammatory responses and protecting the lungs. SFN has been found to be protective in animal models of respiratory disease, including an ARDS model in rabbits [78], and a hyperoxia-induced pulmonary injury model in mice [79]. It also limits RSV replication and virus-induced inflammation in the lungs of wild-type, but not NRF2-null mice [80]. In HIV-1 transgenic rats, SFN increased the GSH levels and the expression of NQO1, and restored the tight junctions between the alveolar epithelial cells [81] and in an *in vitro* model of influenza A infection, SFN reduced both viral cell entry and replication [82]. Additionally, SFN suppresses HCV replication [83] and reduces HSV-1 virion production [29]. Interestingly, SFN inhibits nucleotide-binding oligomerization domain (NOD)-, leucine-rich repeats (LRR)- and pyrin domain-containing protein (NLRP) 1 and 3 inflammasomes (critical innate immune components that shape the host immune homeostasis), and pyroptosis, partly in an NRF2-independent manner [84]. Moreover, an interesting study conducted in smokers (patient cohort with higher risk of lung infections, damage etc) showed that SFN increased the expression of NQO1 in cells of

nasal lavage fluid and, upon infection with live attenuated influenza virus, lowered the levels of IL-6 and viral load [85].

Standardized broccoli extracts and dietary supplements as sources of sulforaphane, as well as encapsulated stabilized sulforaphane (Prostaphane and SFX-01) have been in numerous clinical trials for indications that range from lung diseases to inflammatory diseases which are closely related to COVID-19 pathophysiology. These include chronic obstructive pulmonary disease (COPD), asthma, allergy, rhinitis, ageing, diabetes mellitus, *Helicobacter pylori* infection, and subarachnoid haemorrhage (Table 1). The clinical trials provide extensive pharmacokinetics, pharmacodynamics, safety and efficacy information [77] that can be extrapolated to COVID-19. Notably, most of these trials have recommended cruciferous-free diets during the study period in order to minimize baseline noise and be able to detect accurately the plasma and urinary levels of sulforaphane and its metabolites [86].

Bardoxolone methyl (CDDO-Me) and omaveloxolone (RTA-408)

Semi-synthetic pentacyclic triterpenoids derived from the natural product oleanolic acid represent the most potent NRF2 activators known to date, with activities in the sub- to low-nanomolar concentration range [87]. An early study highlighted that the anti-inflammatory and NRF2 inducer potencies among 18 triterpenoids were linearly correlated over 6 orders of magnitude of concentration, suggesting that the two processes were mechanistically linked [88]. Some triterpenoids isolated from *Ganoderma lucidum* have been found to be potential inhibitors of the NS2B-NS3 protease of DENV [89]. A study conducted in 2003 after the SARS outbreak [90] found that glycyrrhizin, a triterpenoid from liquorice roots, inhibited replication of SARS-CoV in two clinical isolates of coronaviruses from patients with SARS. The potential effect of glycyrrhizin on NRF2 was not studied; however glycyrrhizin has been shown to

activate NRF2 in other settings [77, 91], and it is plausible that at least part of the observed antiviral effect was due to NRF2 activation.

Bardoxolone methyl (CDDO-Me), a semi-synthetic pentacyclic triterpenoid, has been shown to possess a broad-spectrum anti-inflammatory activity against both DENV and zika virus (ZIKV) [92]. In preclinical models, bardoxolone methyl alleviated LPS-induced acute lung injury through NRF2-dependent suppression of inflammation and oxidative stress [93]. The antiviral properties of bardoxolone methyl have also been shown in cell culture models of HBV-, HCV-, and HSV1-mediated infections, where it reduced the levels of intracellular HBV RNA pregenome, suppressed HCV genome replication [94], and the production of HSV1 virions [29]. Bardoxolone methyl and a closely related analog, omaveloxolone (RTA-408) are currently in clinical trials for various indications, where inflammation and oxidative stress underlie disease pathogenesis, including for pulmonary hypertension, pulmonary arterial hypertension, and ocular inflammation (Table 1) [95].

It is important to note that even though NRF2 is the primary mediator, additional factors contribute to the anti-inflammatory effects of SFN, bardoxolone methyl and omaveloxolone (RTA-408). Thus, SFN inhibits NF-κB [96], inhibitor of NF-κB kinase subunit beta (IKKβ) [97] and STAT3 [98], whereas bardoxolone methyl inhibits IKKβ [99], and the activation of STAT3 and STAT5 [100]. Overall, the combined NRF2-activating and anti-inflammatory effects of these compounds culminate in a highly robust cytoprotection. Hence these compounds can be potentially explored in management of symptoms in COVID-19.

NRF2 activators versus other anti-inflammatory approaches to COVID-19

It can be suggested that the exacerbated inflammation observed in COVID-19 patients can be treated with anti-inflammatory drugs such as corticosteroids and **nonsteroidal anti-inflammatory drugs (NSAIDs)**. Indeed, the RECOVERY (Randomised Evaluation of COVid-19 thERapY) trial, a randomized multi-center clinical trial in COVID-19 patients from National Health Service (NHS) hospitals in the UK, found that low-dose dexamethasone, a corticosteroid, reduced mortality in ventilated patients and in patients receiving oxygen only, although it had no effect in patients not receiving respiratory support [101].

Although results with NSAIDs are not conclusive in people with COVID-19 [102], ibuprofen, an NSAID, has been shown to impair neutrophil function, their recruitment to the inflammatory site, and the resolution of inflammatory processes in patients with pneumonia [103]. However, ibuprofen is associated with higher rates of nephrotoxicity [104], cardiovascular disease, and stroke [105] and appears to increase the risk for these outcomes in ARDS [106]. A significant difference between NSAIDs and NRF2 is that NRF2 elicits a much more integrated regulation of the inflammatory response, being necessary for both its execution and resolution. Furthermore, by regulating the endogenous cytoprotective systems, NRF2 may have a more physiological role in achieving a balance between beneficial and adverse effects of inflammation.

Another alternative to conventional anti-inflammatory drugs that holds great promise in COVID-19 is the use of drugs that target cytokines involved in cytokine storm in COVID-19. This includes targeting IL-6 and IL-1 signaling [107]. Tocilizumab (humanized monoclonal antibody against the IL-6 receptor) and anakinra, a recombinant human IL-1 receptor antagonist, are being repurposed and studied in COVID-19 [108, 109]. Use of NRF2 activators represents an excellent alternative or

parallel to these approaches as it is known that NRF2 inhibits IL-6 and IL-1β gene expression [21].

Conclusions and Future Perspectives

In the past few months, COVID-19, a disease caused by a novel coronavirus SARS-CoV-2, has had a tremendous health and socio-economic impact on a global scale. Here we propose a potential anti-inflammatory therapy based on pharmacological targeting of transcription factor NRF2. We envision that the benefits of pharmacological activation of NRF2 in the context of SARS-Cov-2 infection will be three-fold: 1) increasing fitness and providing protection to the host cell; 2) promoting the anti-inflammatory phenotype during macrophage activation and thus preventing uncontrolled production of proinflammatory cytokines and pyroptosis; 3) inhibiting viral propagation. Notably, unlike direct antioxidants, such as vitamin C, which are short-lived (minutes to hours) and consumed in the process of ROS-scavenging, the antioxidant and cytoprotective effects of NRF2 activation are long-lasting and persist for several days after inducer elimination [110, 111]. This is because they are mediated by enzymes, which in contrast to small molecules, have long half-lives [11] and are not consumed, but are regenerated during the reactions which they catalyze [112].

Most NRF2 inducers, including DMF, sulforaphane and bardoxolone methyl, are electrophiles that modify cysteine sensors of KEAP1 and inactivate its repressor function. Concerns of using electrophilic NRF2 activators include: 1) possible toxicity at high doses. Electrophiles react with GSH and thus, at high doses, may cause GSH depletion; indeed an electrophilic metabolite is responsible for the hepatotoxicity of high doses of acetaminophen [113], 2) possible perturbations in redox signaling due to persistent NRF2 activity. Both concerns can be resolved by careful selection of dose

and dosing regimen in the design of clinical trials, such as including periods of NRF2 activation only during times of active disease with the aim to restore redox balance and resolve inflammation. Moreover, experimental evidence suggest that the maximally tolerated dose of sulforaphane (and other electrophiles) is not necessarily the most effective [114]. This realization is driving research that aims to identify non-electrophilic NRF2 activators, which inhibit binding to KEAP1, and where promising candidates are emerging [71, 115, 116].

An area of potential interest for developing COVID-19 candidate drugs in the NRF2 pathway would be to target transcriptional repressor BTB domain and CNC homolog 1 (BACH1) which shows increased levels as NRF2 activation declines with age (a risk factor in COVID-19) [18]. The identification of compounds with BACH1-inhibiting activities is desirable as these might be candidate drugs against COVID-19.

Collectively, the discussed research strongly suggests that NRF2 activation holds promise as a strategy against COVID-19. However, before implementing this strategy, it is desirable to answer several important questions (see **Outstanding Questions**). For example, the finding that SARS-CoV-2 inhibits NRF2 [33] indicates that the virus deprives the host cells from an essential cytoprotective pathway, and it will be critical to determine how and when during the process of the viral infection does this occur, and what is the underlying mechanism. It is presently unclear whether NRF2 can contribute to metabolic reprogramming and adaptation of macrophages and T cells, and whether it affects their effector functions during the course of COVID-19. It will also be important to establish if pharmacological activation of NRF2 can suppress entry of SARS-CoV-2 into the host cell, how NRF2 activators reduce the replication of SARS-CoV-2, and whether this effect depends on NRF2, HO-1 or the broader network of proteins regulated by NRF2.

Nonetheless, the wealth of safety and efficacy information for NRF2 activators such as sulforaphane and bardoxolone methyl, that are already in advanced clinical trials for other indications, provides a clear route for their testing in randomized clinical trials in patients with COVID-19. If successful, this therapeutic strategy could be mobilized rapidly in order to improve recovery and decrease the need for mechanical ventilation in patients with severe COVID-19, relieving the enormous strain that is currently being experienced by intensive care units worldwide.

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DISCLAIMER STATEMENT

A.T. D-K. is on the Scientific Advisory Board of Evgen Pharma and is a consultant for Aclipse Therapeutics and Vividion Therapeutics. AC is consultant for Aclipse Therapeutics.

RESOURCE

i) https://www.clinicaltrials.gov/

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TABLE

Table 1. Selected clinical trials of sulforaphane and its encapsulated variant sulfodarex (SFX-01), bardoxolone-methyl and its structural analog omaveloxolone (RTA-408), the four NRF2 activators that target KEAP1.

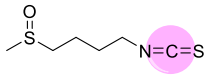
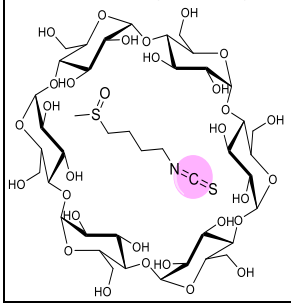
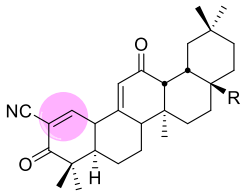
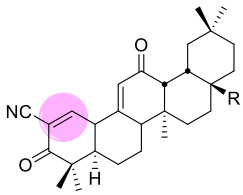
Compound	**Disease**	**Clinical trial**	**ClinicalTrials.gov[i] Identifier**
Sulforaphane 	Healthy	Phase I	NCT01008826
		Phase I	NCT02023931
	Chronic obstructive pulmonary disease (COPD)	Phase II	NCT01335971
	Asthma	Phase I	NCT01845493
		Phase I	NCT01845493
		Phase I/II	NCT01183923
	Aging	Phase II	NCT03126539
	Allergic Rhinitis	Phase II	NCT02885025
	Helicobacter Pylori infection	Phase IV	NCT03220542
	Type 2 diabetes mellitus	Phase II	NCT02801448
Sulforadex (SFX-01) 	Subarachnoid haemorrhage	Phase II	NCT02614742

Table 1 (continued).

Compound	Disease	Clinical trial	ClinicalTrials.gov Identifier
Bardoxolone-methyl (CDDO-Me)  R = CO_2Me	Pulmonary hypertension	Phase III	NCT03068130
		Phase III	NCT02657356
	Renal insufficiency, Type 2 diabetes mellitus	Phase II	NCT01053936
	Type 2 diabetic nephropathy Chronic Kidney Disease	Phase II	NCT00811889
	IgA nephropathy Chronic Kidney Disease associated with type 1 DM Focal segmental glomerulosclerosis Autosomal dominant polycystic kidney	Phase II	NCT03366337
	Chronic Renal Insufficiency Type 2 Diabetes Mellitus	Phase III	NCT01351675
	Liver Disease	Phase I/II	NCT00550849
	Hepatic impairment Healthy	Phase I	NCT01563562
	Alport syndrome	Phase II/III	NCT03019185
Omaveloxolone (RTA-408)  R = $NHCOCF_2Me$	Inflammation and pain following ocular surgery	Phase II	NCT02065375
	Corneal endothelial cell loss Ocular pain Ocular inflammation Cataract surgery	Phase II	NCT02128113
	Radiation dermatitis in breast cancer	Phase II	NCT02142959

Note: The pink circle on the chemical structures indicates the electrophilic carbon, which undergoes nucleophilic attack by cysteine 151 of KEAP1.

FIGURE LEGENDS

Figure 1. Putative viral cycle of SARS-CoV2 highlighting the points of potential crosstalk with NRF2 activation. Steps 1 to 7 depict the different steps of viral cycle. **1)** Binding of the viral spike (S) protein to ACE2 leads to its entry. NRF2 has been shown to repress *ACE2* gene expression in rats [43]. **2)** The viral nucleocapsid is uncoated in the cytoplasm of the host cell. **3)** Translation of the +ssRNA and cleavage into specific viral proteins. With the presence of viral RNA inside the host cells, DNA/RNA sensor cGAS, which signals through the adaptor STING [117], induces the expression type I and III interferons (IFNs). NRF2 represses IFN production by downregulating STING expression [56]. **4)** Replication of the viral genome. NRF2 induces the expression of HO-1, generating Fe^{2+} which can bind to the divalent metal-binding pocket of the RNA-dependent RNA polymerase (RdRp) of SARS-CoV2 and inhibit its catalytic activity [63, 64]. **5)** Translation of structural proteins. Host defense is conducted by double-stranded RNA-activated protein kinase R (PKR), which phosphorylates eIF2 and inhibits protein translation. PKR also phosphorylates p62, thus activating NRF2 upon removal of its repressor KEAP1 by autophagy [118]. Inhibition of protein translation in turn activates the unfolded protein response (UPR). PERK, a crucial Ser/Thr protein kinase in UPR signaling, phosphorylates NRF2, resulting in its stabilization and increased transcriptional activity [49]. **6)** Virions assembly. **7)** Release of viral particles. **Abbreviations used:** ACE2: angiotensin converting enzyme 2, eIF2: eukaryotic initiation factor 2, ERGIC: endoplasmic reticulum-Golgi intermediate compartment, HO-1: heme oxygenase 1, IFN: interferon, KEAP1: Kelch-like ECH-associated protein 1, NRF2: nuclear factor erythroid 2 p45-related factor 2, PERK: PKR-like endoplasmic reticulum kinase, PKR: protein kinase R, STING: stimulator of interferon genes. Figure generated in Biorender (https://biorender.com/).

Figure 2. Antiviral activity of HO-1, a target of NRF2. HO-1 catalyzes the degradation of heme into carbon monoxide (CO), Fe^{2+} and biliverdin. Free Fe^{2+} is expected to bind to the highly conserved divalent metal-binding pocket of RNA-dependent RNA polymerase (RdRp). Carbon monoxide activates soluble guanylyl cyclase (sGC) to generate cGMP thus activating protein kinase G (PKG), which inhibits NAPDH oxidases (NOX), preventing an increase in reactive oxygen species (ROS). By inhibiting 3CLpro and PLpro SARS-CoV-2 proteases, biliverdin is expected to suppress the proteolytic maturation of viral polypeptides. Heterodimerization of HO-1 with IRF3 facilitates the phosphorylation and nuclear translocation of IRF3 and induction of type I IFN gene expression. **Abbreviations used:** cGMP: cyclic guanosine monophosphate, HO-1: heme oxygenase 1, IFN: interferon, ISRE: interferon-sensitive response element, IRF3: interferon regulatory factor 3, NRF2: nuclear factor erythroid 2 p45-related factor 2. Figure generated in Biorender (https://biorender.com/).

GLOSSARY

Acute respiratory distress syndrome (ARDS): A type of respiratory failure characterized by rapid onset of widespread inflammation in the lungs which impairs their ability to exchange oxygen and carbon dioxide.

Angiotensin converting enzyme 2 (ACE2): A transmembrane receptor protein found in lungs, arteries, heart, kidney, and intestine, which serves as the main entry point into cells for SARS- CoV and SARS-CoV2.

Antioxidant Response Elements (ARE): Specific DNA sequences that are present in the promoter regions of the several genes that encode cytoprotective proteins and detoxification enzymes.

Bioavailability: The proportion of a drug or other substance, which when introduced into the body, enters the circulation and is able to have an effect.

Electrophile: A chemical species, which is attracted to an electron-rich center. It is chemically reactive, and by accepting an electron pair, binds to a nucleophile.

Granulocytosis: Increase in number of granulocytes (basophils, eosinophils and and neutrophils) in the peripheral blood.

Glutathione (GSH): Glutathione is the most abundant thiol in animal cells, with more than 90% of the total glutathione in the reduced form (GSH), with the remainder in the disulfide form (GSSG). Increased GSSG/GSH ratio is indicative of oxidative stress.

Macrophage Activation Syndrome (MAS): A potentially fatal complication of rheumatic diseases, which is characterized by high fever and can be associated with hemorrhages, damage to the liver, kidneys and the central nervous system, and may lead to multiple organ failure.

NAD(P)H quinone oxidoreductase (NQO1): Homodimeric FAD-binding protein, which catalyzes the obligatory two-electron reduction of quinones to hydroquinones

thus preventing redox cycling and glutathione depletion. NQO1 is a classical NRF2-regulated gene and is used as a marker of NRF2 transcriptional activity.

Nonsteroidal anti-inflammatory drugs (NSAIDs): Inhibitors of the enzyme activity of cyclooxygenases (COX-1 or COX-2), which catalyze the biosynthesis of prostaglandins (involved in inflammation) and thromboxanes (involved in blood clotting).

Oxidative stress: An imbalance between oxidants and antioxidants in favour of the oxidants, leading to a disruption of cellular redox signaling and damage of proteins, lipids and DNA.

Reactive oxygen species (ROS): An unstable oxygen-containing molecule that is formed by redox reactions or by electronic excitation.

STimulator of INterferon Genes (STING): An adaptor protein associated with the endoplasmic reticulum, which is essential for transcription of host defense genes, including type I interferons (IFNs) and proinflammatory cytokines, following recognition of the presence of aberrant DNA species or cyclic dinucleotides (CDNs) in the cytosol.

Unfolded protein response (UPR): A homeostatic signaling network which is activated following endoplasmic reticulum stress and results in functional recovery of the organelle.

Outstanding Questions

- How does SARS-CoV-2 downregulate NRF2 in the host cells, and does this depend on the stage of the viral infection?

- Does NRF2 contribute to metabolic reprogramming and adaptation of macrophages and T cells, and does it affect their anti-inflammatory functions during the course of COVID-19?

- Can pharmacological activation of NRF2 decrease entry of SARS-CoV-2 into the host cell?

- Do all NRF2 activators reduce replication, virion production, or do they affect other stages of propagation of SARS-CoV-2?

- If they do, does this effect depend on NRF2 and/or HO-1?

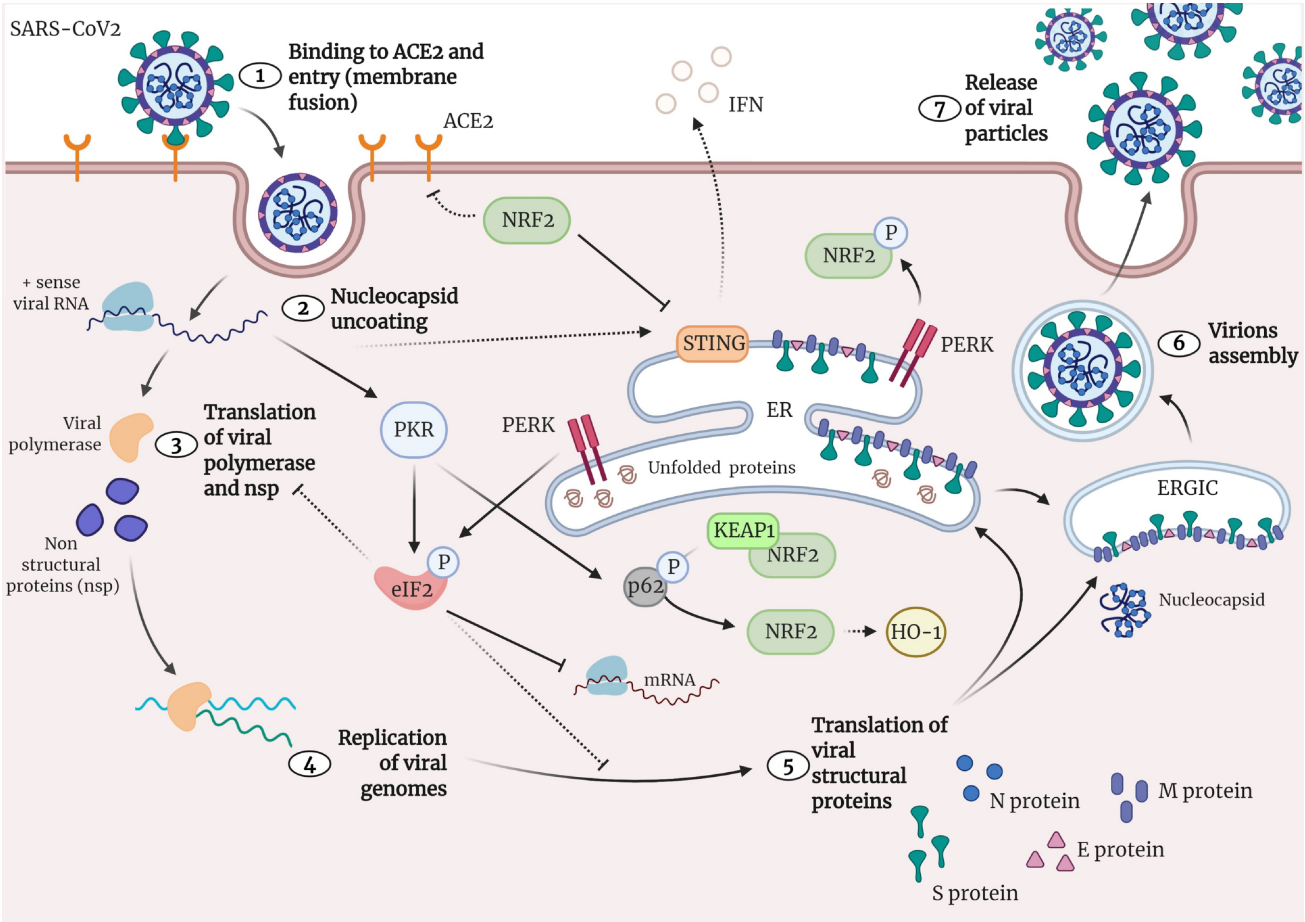
Highlights

- The host inflammatory response is a critical determinant of disease outcome and correlates with disease severity in SARS-CoV-2-induced infection, for which there is no treatment to date.

- Activation of transcription factor Nuclear factor erythroid 2 p45-Related Factor 2 (NRF2) promotes resolution of inflammation and in parallel, restores the cellular redox and protein homeostasis, and facilitates tissue repair.

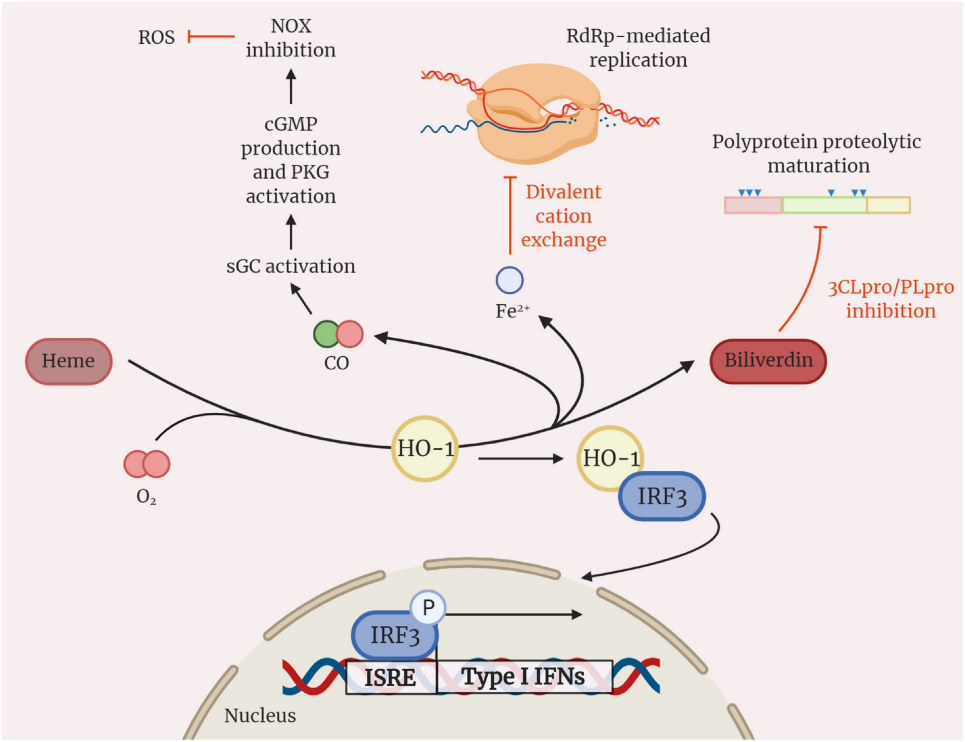
- NRF2 can be activated by pharmacological inducers that target Kelch-like ECH-associated protein 1 (KEAP1), the principal negative regulator of NRF2.

- The available information on pharmacokinetics, pharmacodynamics, safety and efficacy for the NRF2 activators sulforaphane and bardoxolone methyl (currently in advanced clinical trials for other disease indications) in humans, makes them excellent candidates for testing in randomized clinical trials in COVID-19.



Trends in Pharmacological Sciences

Figure 1



Trends in Pharmacological Sciences

Figure 2